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The passage of digesta, particle size, and *in vitro* fermentation rate in the three-toed sloth *Bradypus tridactylus* (Edentata: Bradypodidae)

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(With 6 figures in the text)

The digestive physiology of six three-toed sloths (*Bradypus tridactylus*) fed exclusively on *Cecropia palmata* foliage was studied. The mass of digesta in the gut was between 17 and 37% of body mass. This was between 133 and 282% of that expected in an animal of this size, based on published allometric equations. The concentration of total short chain fatty acids in the stomach was similar to that in the fermentative regions of other foregut fermenting herbivores but the rate of fermentation measured *in vitro* was very slow (6–12 mmol.l^{-1}.h^{-1}) and substantially lower than that observed using similar techniques in other small foregut fermenters.

The overall (dose to excretion) mean retention time of particulate and solute digesta markers was about 150 h. Most of this (73%) occurred in the stomach but a substantial proportion (17%) could be attributed to the storage of faeces in the rectum.

The slow rate of passage of digesta through the gut together with the slow rate of fermentation in the stomach is not typical of small foregut fermenting herbivores. However, such a pattern is feasible in *Bradypus tridactylus* because of the large volume of digesta retained in the gut and the very low metabolic rate of these mammals.

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Introduction

The size of vertebrates which are both arboreal and folivorous is constrained in two ways. It is advantageous for them to be small because they can move easily through the forest canopy and can feed on young leaves at outer branchlets. However, tree foliage is a poor quality food because it contains little protein but is rich in lignified fibre (Cork & Foley, 1991) and many studies (e.g. Demment & Van Soest, 1980) have concluded that such a diet is best used by large animals. Evidently the opposing directions of these constraints allow relatively few solutions and arboreal folivores occupy a narrow range of body masses. The smallest are about 700 g (marsupial ringtail possums (*Pseudocheirus* sp.) and greater gliders (*Petauroides volans*) and the sportive lemur (*Lepilemur* sp.), while the largest are only 12–15 kg (koalas (*Phascolarctos cinereus*) and some colobid primates, e.g. *Colobus*). Some species are foregut fermenters while others are hindgut fermenters.

No arboreal folivore can rely on the fermentation of plant cell walls as its principal source of energy because the yield of energy from fermentative digestion of fibrous tree leaves is too slow in relation to the high energy requirements of these small mammals (Cork & Foley, 1991). Foregut fermentation should be even less favourable for small animals since soluble constituents are fermented and thus used at considerably lower efficiency than if they were directly absorbed.

A recent review of the nutritional ecology of arboreal folivores (Cork & Foley, 1991) has challenged the long-held view that foregut fermenters are better able to use a diet of tree foliage than are hindgut fermenters. The most folivorous mammals are hindgut fermenters which can separate digesta in the caecum and rapidly excrete fibrous material (koala, greater glider and sportive lemur; Cork & Foley, 1991). In contrast, nearly all foregut-fermenting arboreal herbivores eat considerable amounts of fruit and seed.

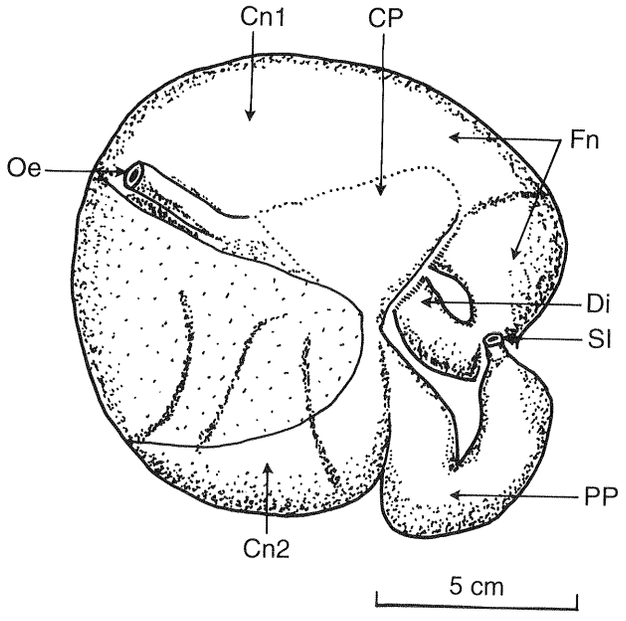

FIG. 1. Stomach of the three-toed sloth *Bradypus tridactylus*. Nomenclature follows Langer (1988) except that the connecting pouch was divided into two separate areas. Abbreviations are as follows: Oe, oesophagus; Di, diverticulum; Fn, fundus; CP, central pouch; Cn1, cranial part of connecting pouch; Cn2, caudal part of connecting pouch; PP, prepyloric stomach; SI, small intestine.

Three-toed sloths challenge these generalizations because they are small (2–4·5 kg), entirely folivorous (Montgomery & Sunquist, 1978) and are foregut fermenters. Cork & Foley (1991) suggested that the very slow metabolic rate of sloths (McNab, 1978; Nagy & Montgomery, 1980) may allow a more extensive use of foliage than would otherwise be possible for such a small foregut fermenter.

To test these ideas, we measured aspects of the digestive processes of *Bradypus tridactylus* captive in French Guiana. The lack of previous studies meant that our investigations were broad and so include data on the volume of the digestive tract, passage of markers of particulate and liquid digesta, distribution of particle sizes in the gut and measurements of fermentation rate *in vitro*. A schematic representation of the stomach of *Bradypus tridactylus* is shown in Fig. 1.

Materials and methods

Animals

Six 3-toed sloths (*Bradypus tridactylus*) were obtained from secondary forest near Cayenne, French Guiana (see Charles-Dominique *et al.* (1981), for further details of habitats and fauna). The animals were held in individual cages (2 m high × 1·5 m × 1·5 m), maintained in a room at ambient temperature but without access to direct sunlight. Each cage was supplied with branches for the animals to rest on. The animals were fed solely on leaves of *Cecropia palmata* which were cut fresh each day at dusk. Samples of the foliage offered to each animal during the course of the experiments were bulked. This bulk sample contained 92·3% organic matter, 2·55% nitrogen, 39·1% neutral detergent fibre, 14·0% acid-lignin and 19·8 kJ/g dry matter of gross energy.

The sloths defecated and urinated only when provided with bowls of water on the cage floor. These bowls were modified by covering the water surface with fine wire mesh which was attached to the sides of the bowl with elastic bands. This allowed the sloth the sensation of the water when it sat on the mesh but kept the faeces from being immersed in water after the animal had finished defecating. Urine could not be collected reliably although several samples were collected opportunistically.

We maintained each animal in captivity for at least 3 weeks before commencing the experimental procedures. Although body mass fluctuated throughout the time that the animals were in captivity, this was largely due to irregular defecations and all animals increased their initial body mass.

Experimental protocol

Digesta markers

The very irregular defecations of 3-toed sloths (reportedly only 7–10 days; Montgomery & Sunquist, 1978) precluded the use of total faecal collections as a means of estimating parameters of digesta passage. We therefore used a method which involved measuring the amount of marker in each segment of the digestive tract after the death of the animal. This is equivalent to method PKD described by Warner (1981). In order to limit the number of animals in the experiments, we used 6 marker substances in each animal. The particulate digesta phase was marked with $YbCl_3$, $LaCl_3$ and $SmCl_3$. These rare earth elements have a strong affinity for particulate digesta and are widely used as markers of particulate phase digesta in other foregut fermenters (Ellis *et al.*, 1982). The 3 rare earth elements were purchased as their oxides and dissolved in 1M HCl. Each dose consisted of 0·5 g of the rare earth element.

The solute digesta phase was marked with 3 complexes of ethylenediamine tetra-acetic acid (EDTA). These were Cr-EDTA, Co-EDTA and Fe-EDTA. Cr-EDTA and Co-EDTA have been widely used for this purpose in a range of animal species. Fe-EDTA has not been widely used but Teeter & Owens (1983) found that all 3 complexes gave similar estimates of the kinetics of solute digesta in the rumen of steers.

Polyethylene glycol (PEG) was precipitated by aqueous and acetone extracts of leaves and faeces and was thus unsuitable for use as a solute marker. Cr-EDTA was prepared according to the method of Binnerts, van't Klooster & Frens (1968), and the sodium salt of Co-EDTA was prepared as described by Uden, Collucci & van Soest (1980). We were unable to prepare Fe-EDTA using the method described by Teeter & Owens (1983). However, we were able to prepare the ammonium salt of Fe-EDTA using the technique of Brintzinger, Thiele & Muller (1943). Each animal received 0·5 g of each complex.

Markers were given randomly. Thus, animal 1 received $YbCl_3$ and Cr-EDTA at Time 1 (i.e. 192 h before death), $SmCl_3$ and Co-EDTA at Time 2 (60 h before death) and $LaCl_3$ and Fe-EDTA 24 h before death. The other animals received random combinations of the particulate and solute markers at different times before death. We were thus able to make observations of the excretion of markers at 18 times ranging from 6 h to 288 h before death.

All markers were dosed in liquid form. A paediatric feeding tube was lubricated with glycerol and passed down the oesophagus of the animal into the stomach. Water (1·0 ml) was injected to ensure that the tube was in place in the stomach. The particulate marker (1·0 ml) was then injected followed by more water (1·0 ml) and then the solute marker (1·0 ml). All animals were observed to eat within the hour before and after dosing. All faeces were collected and the time of defecation noted from the time of the initial marker dose until death.

Collection of samples

Animals were weighed and sedated with an intramuscular injection of sodium pentobarbitone and the rectal temperature was measured. The animals were then killed with a further injection of sodium pentabarbitone. The gut was divided into 10 segments using clamps and ligatures. A small incision was made in the wall of each segment and the pH measured (within 8 min of death) with a pH meter. Each gut segment was then weighed to the nearest gram. Samples of the contents were then taken for determination of the dry matter content, concentrations of the 6 markers, short chain fatty acids (SCFA) and the distribution of digesta particle size. When there were sufficient digesta, the remainder of the fundus and connecting pouch was transferred to warm glass jars, gassed with oxygen-free CO_2, and incubated at the animal's body temperature for 2–2·5 h. The average time from the death of an animal to the commencement of incubations was 38 min. Sub-samples were taken initially at 15 min intervals and then half-hourly so that the rate of change of concentration of SCFA with time could be determined.

Analytical

The dry matter content of samples of digesta and faeces was determined by oven-drying to constant mass at 60 °C. The concentrations of La, Sm, Yb, and Co were determined in each sample by neutron activation and high resolution gamma spectroscopy (Cercasov & Heller, 1980). The concentration of Cr and Fe was measured by atomic absorption spectroscopy. Short-chain fatty acids (SCFA) were extracted from digesta samples by shaking with distilled water followed by high-speed centrifugation (10 min × 15000 g). The supernatant was then acidified with formic acid and analysed by gas-liquid chromatography.

The distribution of digesta particle sizes was determined using the wet-sieving technique described by Evans *et al.* (1973). The total dry matter in each sample was determined by drying a portion to constant mass at 60 °C and the remainder of the sample was partitioned between 6 fractions, using 5 sieves (1000, 500, 250, 125, and 75 μm). The sixth fraction (material passing through the 75 μm sieve) included soluble dry matter.

Calculations

The proportion of each marker that had left a particular section of the gut, for example, the distal colon,

when the animal was killed, was plotted as a function of the time since the marker was dosed. By combining data from all 3 particulate markers and all 3 solute markers, we were able to construct cumulative excretion curves for solute and particulate digesta leaving different parts of the gut. Smooth curves were fitted to these data by eye and mean retention times calculated planimetrically (Warner, 1981).

Examination of the data from the particle size analysis showed that the digesta dry matter was principally composed of coarse and fine fractions. The 6 fractions resulting from the particle size determination were reduced to 3 for analysis. These were large particles (> 500 μm), particles between 500 and 75 μm and the fine particles (<75 μm and soluble dry matter).

The zero-time method of Carrol & Hungate (1954) was used to determine *in vitro* SCFA production. Linear regression was used to fit straight lines to plots of SCFA concentration against incubation time and the slope of these lines was taken as a measure of SCFA production rate.

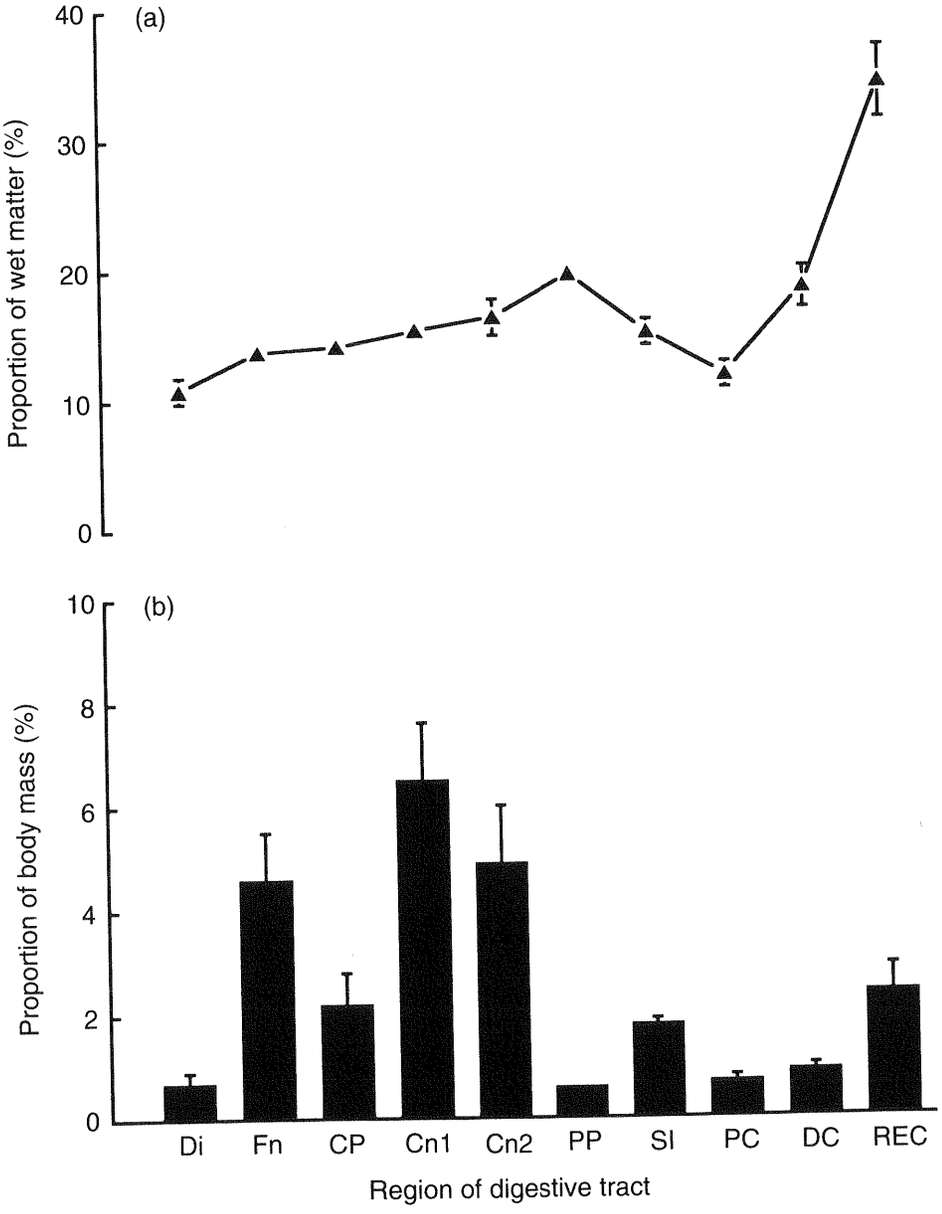

FIG. 2. (a) Dry matter content and (b) mass of digesta in different regions of the digestive tract of *Bradypus tridactylus*. Abbreviations as for Fig. 1, *plus* PC, proximal colon; DC, distal colon; REC, rectum. Mean ± S.E. ($n = 6$).

Results

Food intake and gut contents

All animals gained body mass while in captivity. The range of body mass was 1·2 to 3·9 kg (mean 2·40 kg). The mean dry matter intake was 22 ± 6 g. $kg^{0.75}$. d^{-1} of *Cecropia palmata* foliage.

The proportion of body mass contained in the whole gut ranged from 17 to 37% (Fig. 2b). The majority of the gut contents was found in the stomach (13 to 29%). Stomach contents of smaller animals represented a significantly greater proportion of body mass ($P < 0{\cdot}05$) than that found in the larger animals. Digesta pH (Fig. 3b) was significantly lower in the prepyloric stomach than in

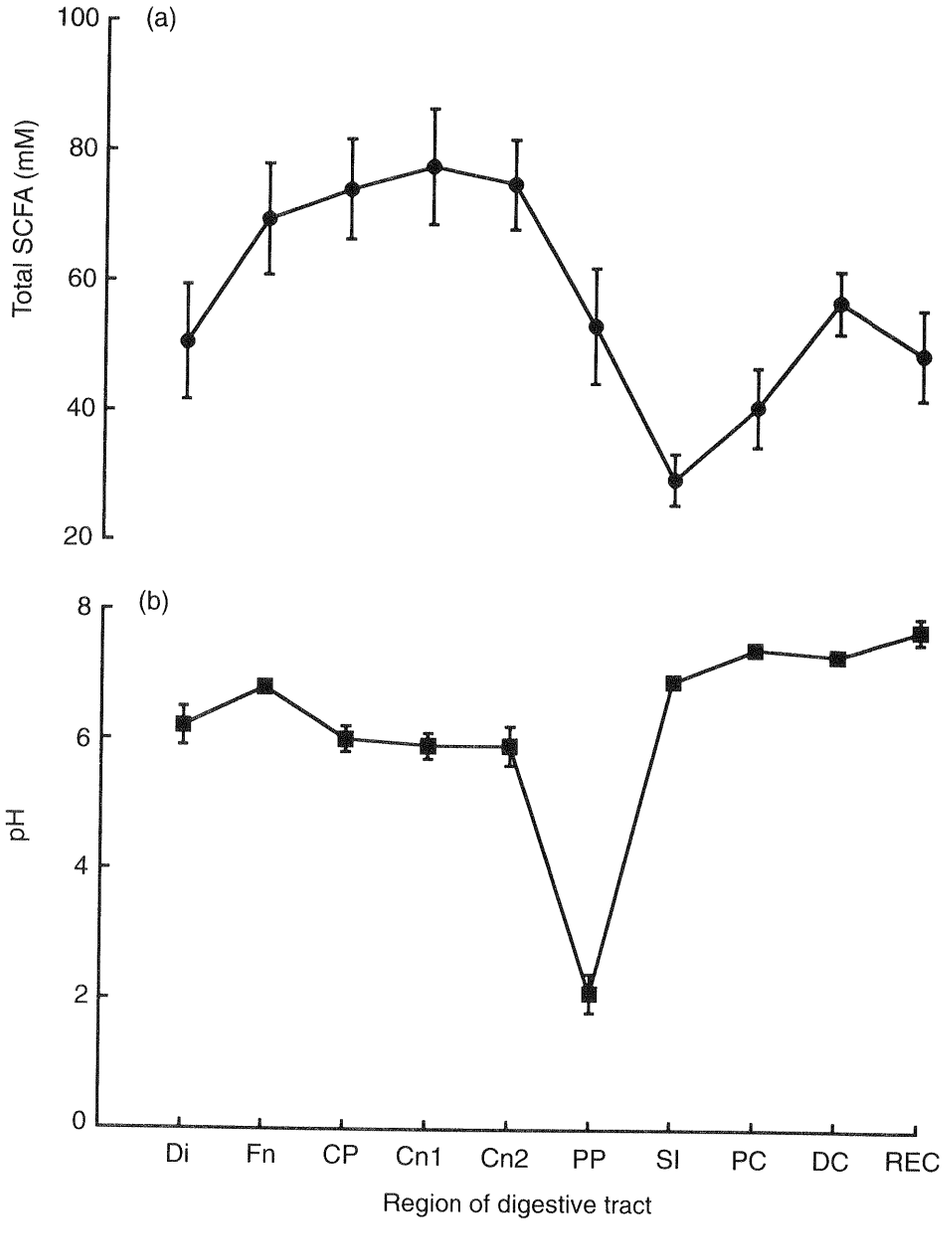

FIG. 3. (a) Concentration of total short-chain fatty acids (SCFA) and (b) pH in different regions of the digestive tract of *Bradypus tridactylus*. Abbreviations as for Figs 1 and 2. Mean ± S.E. ($n = 6$).

the remainder of the gut but there was no significant difference in values in other parts of the stomach.

Fermentation

Details of the concentration of total SCFA and of the individual SCFA are given in Fig. 3a and Table I, respectively. SCFA concentrations were greatest in the stomach and largely mirrored changes in gut pH. However, substantial SCFA was also present in the digesta of the pre-pyloric stomach which probably reflects the poor mixing in this organ. Acetate was the major SCFA present in all regions of the gut but it was notable that branched chain acids (iso-butyric and iso-valeric) were almost undetectable.

Incubations of part of the content of the fundus and connecting pouch at body temperature and under CO_2 showed a linear increase in the concentration of SCFA. Consequently, the slope of these lines was used to estimate SCFA production rates. Five measurements were made of SCFA production in digesta from the connecting pouch but there were sufficient digesta in the fundus of only one animal.

In each instance, the production of total SCFA was slow—between 6 and 12 $mmol.l^{-1}.h^{-1}$ (Table II). Acetate was the principal SCFA produced (52–63% of total SCFA produced in connecting pouch) followed by propionate (30%) and butyrate (9%). Since the production of acetate was lower and that of propionate and butyrate higher than the initial molar proportions in the digesta, it appeared that SCFA were absorbed at a rate directly proportional to their chain length *in vivo*.

Passage of digesta markers

Although total collections of urine were not possible, opportunistic collections from each animal were analysed for markers as an indication of the degree to which they were absorbed

TABLE I

Concentrations (mM) of short-chain fatty acids in digesta in different parts of the gut of ***Bradypus tridactylus****. Mean ± S.E. (in brackets).* $n = 6$

Part of GIT[1]	Short-chain fatty acid			
	Acetic	Propionic	Butyric	Valeric
Di	44·5 ± 7·4	4·3 ± 1·3	1·8 ± 0·8	0 ± 0
Fn	49·9 ± 5·9	12·0 ± 1·3	6·2 ± 1·4	1·4 ± 0·2
CP	53·6 ± 4·7	12·7 ± 1·4	6·5 ± 1·5	1·3 ± 0·4
Cn1	55·5 ± 6·4	14·1 ± 1·4	6·8 ± 1·3	1·4 ± 0·4
Cn2	53·2 ± 4·4	13·7 ± 1·5	6·5 ± 1·1	1·3 ± 0·3
PP	52·4 ± 8·9	0·6 ± 0·2	0·2 ± 0·1	0 ± 0
SI	28·9 ± 4·1	0·4 ± 0·2	0·1 ± 0·0	0 ± 0
PC	40·1 ± 5·9	0·9 ± 0·9	0 ± 0	0 ± 0
DC	51·1 ± 4·0	5·1 ± 1·3	0·7 ± 0·4	0 ± 0
REC	41·0 ± 5·2	5·9 ± 1·4	1·8 ± 0·6	0·2 ± 0·2

[1] Di, diverticulum; Fn, fundus; CP, central pouch; Cn1 cranial part of connecting pouch; Cn2, caudal part of connecting pouch; PP, prepyloric stomach; SI, small intestine; PC, proximal colon; DC, distal colon; REC, rectum.

TABLE II

In vitro production rates of short-chain fatty acids in the stomach of ***Bradypus tridactylus***

Animal	BT1 (Fn)[1]	BT2 (CnP)[2]	BT3 (CnP)	BT4 (CnP)	BT5 (CnP)	BT6 (CnP)
Mass (kg)	3·0	3·0	2·8	1·2	2·3	3·9
Body temp. (°C)	32·0	32·0	34·2	35·1	34·9	34·8
Contents (kg)	0·07	0·216	0·273	0·208	0·279	0·329
SCFA Production ($mmol.l^{-1}.h^{-1}$)						
Acetic	4·0	2·4	7·9	6·5	5·2	2·7
Propionic	1·6	1·6	3·2	3·1	2·7	1·2
Butyric	0·1	0·1	1·0	0·9	1·9	0·3
Other[3]	0·1	0·1	0·4	0·1	0·5	0·1

[1] Fundus
[2] Connecting pouch (Cn1 and Cn2)
[3] Isobutyric, iso-valeric and valeric acids

from the gut. In no case did the concentration of marker in urine exceed 0·2% of the peak marker concentration in the gut or faeces. Although this evidence is limited we concluded that all markers were essentially non absorbable.

Following oral doses, there was no difference in the movement of markers of liquid and solid digesta. This was reflected in both concentrations in digesta from different parts of the gut (Fig. 4) and in estimates of mean retention time (Table III). Initially, markers dispersed into the connecting pouch (Cn1 and Cn2) and it was not until 24 h after dosing that digesta markers accumulated in the diverticulum (Fig. 4). Sixty hours after dosing, a significant part of the marker had already accumulated in the faeces and been excreted and virtually all marker had left the fundus (Fig. 4). The remaining picture was of marker leaving the stomach slowly and accumulating in the rectum over the next 5–7 days (Fig. 4).

The mean retention time of digesta in the gut was estimated from composite excretion curves. These are shown in Fig. 5 for four regions of the gut. The composite method adopted only allowed reliable estimates of mean retention time in the stomach regions caudal to Cn2. From

TABLE III

Mean retention time of markers of solute and particulate digesta in the gut of ***Bradypus tridactylus***[1]*.(h)*

	Cumulative MRT[2]		Compartment MRT[3]	
Site in GIT	Solute	Particulate	Solute	Particulate
Connecting Pouch	87·3	84·6	–	–
Prepyloric Stomach	94·1	90·0	6·8	5·4
Small Intestine	101·8	98·1	7·7	8·1
Proximal Colon	116·4	115·2	14·6	17·1
Distal Colon	125·7	121·8	9·3	7·6
Rectum	151·4	146·8	25·7	25·0

[1] Values are derived from a single cumulative excretion curve constructed from data from six animals and therefore no estimate of variance can be made.
[2] Overall mean retention time from mouth to excretion from that part of the gut.
[3] Estimated mean retention time within that part of the gut.

this point it was assumed that there was no retrograde flow of digesta into more cranial parts of the stomach.

Estimates of the mean retention time of markers in different parts of the gut are given in Table III. The overall (dose to excretion) mean retention time for both particle and liquid markers was 147–152 h. Most of this was attributable to retention in the stomach—notably regions cranial to Cn2. Retention in the rectum accounted for 17% of the overall mean retention time.

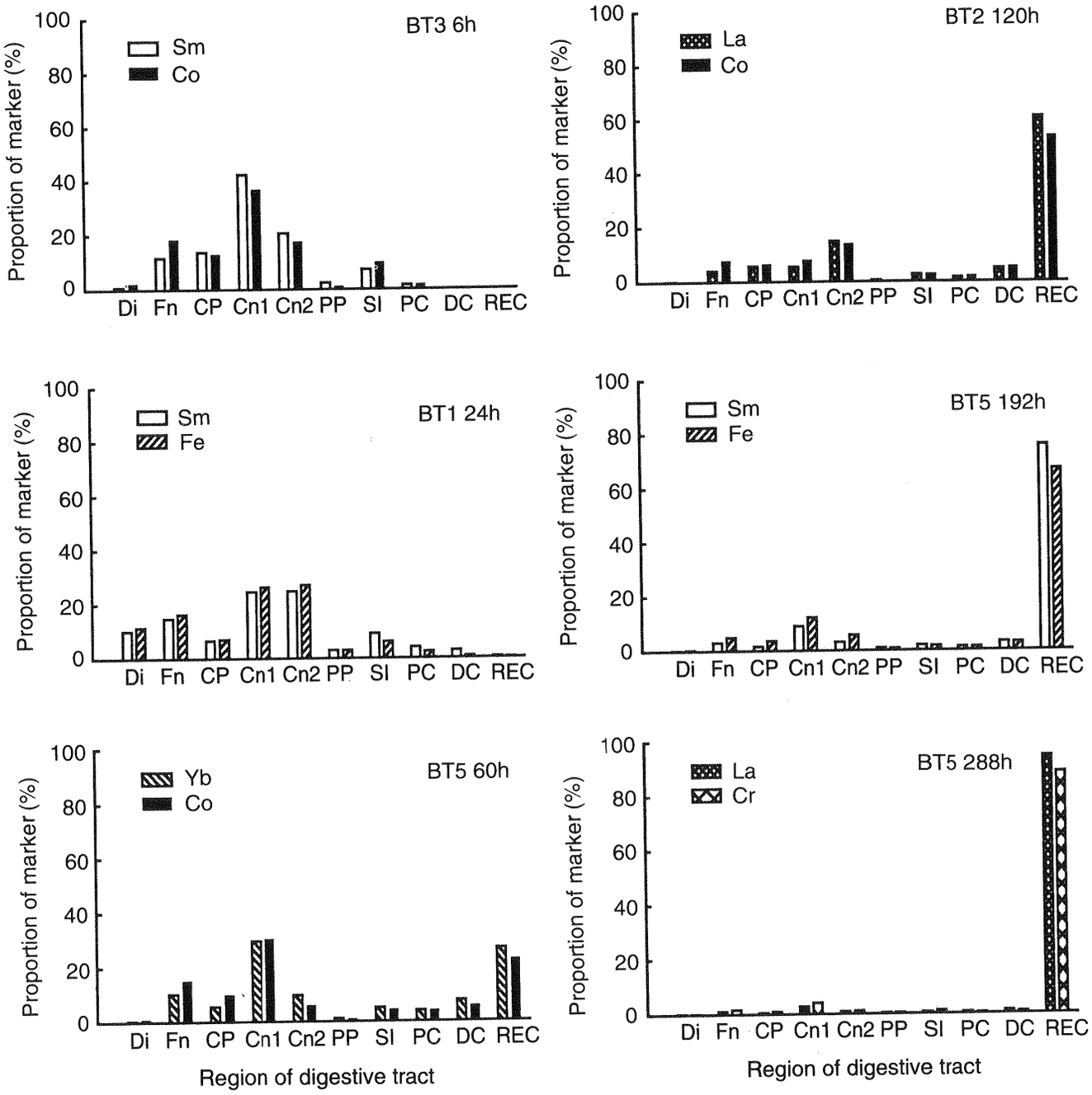

FIG. 4. The distribution of markers of particulate (La, Sm, Yb) and fluid (Fe-EDTA, Co-EDTA, Cr-EDTA) digesta fractions in different regions of the gut of *Bradypus tridactylus* at varying times after an oral dose. The figures shown illustrate the range of times and combinations of markers that were used. Abbreviations as for Figs 1 and 2.

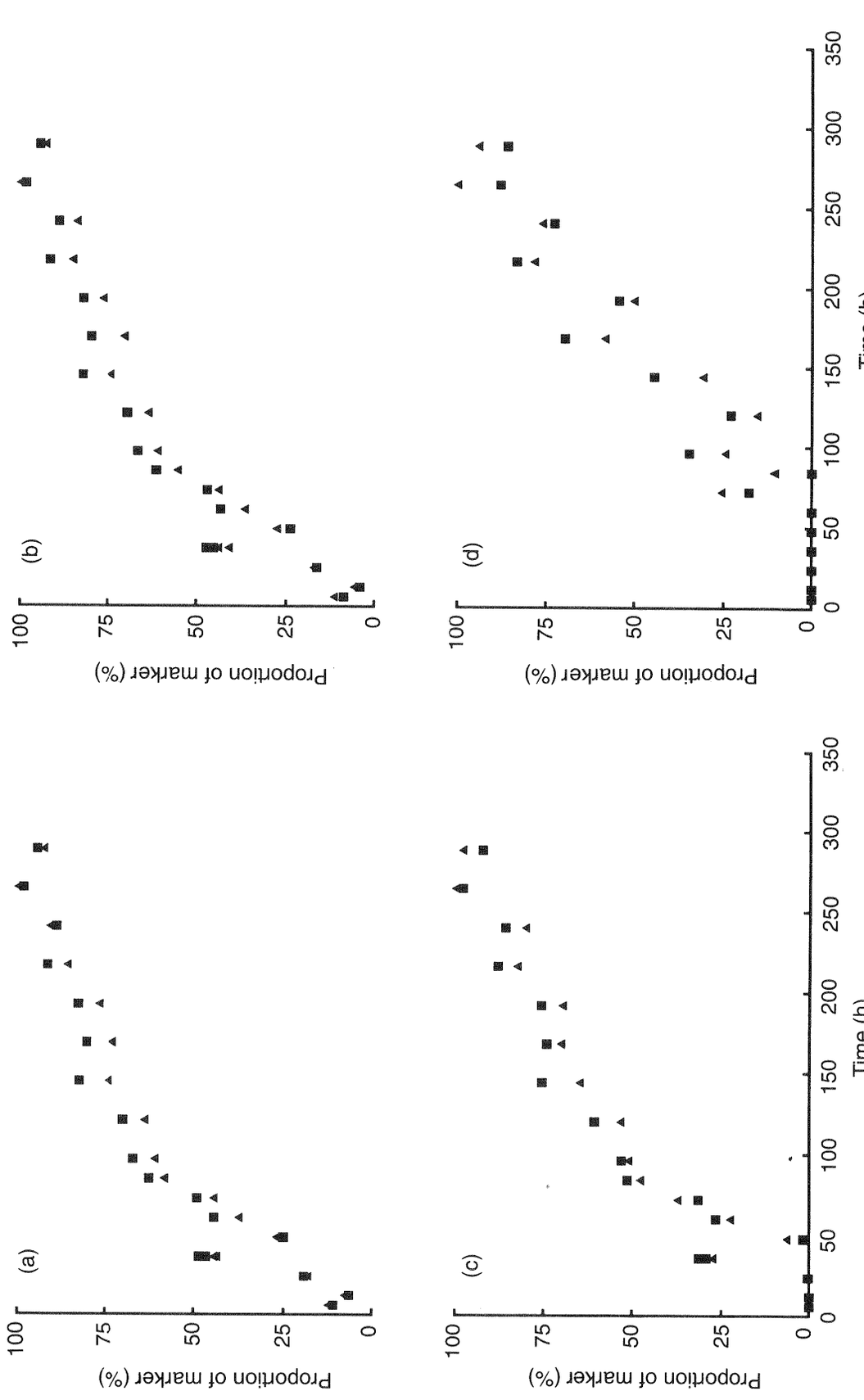

FIG. 5. Excretion curves of markers of solute (▲) and particulate (■) digesta from four regions of the digestive tract of *Bradypus tridactylus*: (a) marker distal to cranial portion of connecting pouch (Cn1); (b) marker distal to prepyloric stomach; (c) marker distal to distal colon; (d) marker distal to rectum (i.e. excreted). The curves have been constructed from data for six individual animals. It was assumed that there was no retrograde flow of marker from regions distal to Cn2 (caudal part of connecting pouch). The mean retention time of solid and liquid phase markers was derived from the area bounded by each curve.

Digesta particle size

The distribution of coarse (>500 μm), intermediate (500–75 μm) and fine (<75 μm including solutes) digesta is shown in Fig. 6. In all parts of the gut with the exception of the pylorus, the fine particle fraction formed the bulk of the digesta. In the prepyloric stomach, there were significantly more ($P < 0{\cdot}05$) coarse particles than in other regions of the gut. Conversely, in the small intestine there were significantly fewer coarse particles than in the stomach.

Discussion

The most striking feature of the digestive tract of *Bradypus tridactylus* was the volume of digesta in the stomach. The values observed (17–37% of body mass) were similar to those measured in earlier studies (Wislocki, 1928; Britton, 1941). A more useful comparison can be obtained by using the allometric relationship between body mass and mass of gut contents derived by Demment & Van Soest (1980). On this basis, three-toed sloths contained between 133 and 282% of the predicted mass of digesta for an animal of that body mass. Even without more detailed investigations, it is clear that such a large gut capacity provides this small animal the luxury of prolonged retention of digesta and slow fermentation rate.

Fermentation

The concentration and molar proportions of SCFA in the stomach of *Bradypus tridactylus* are

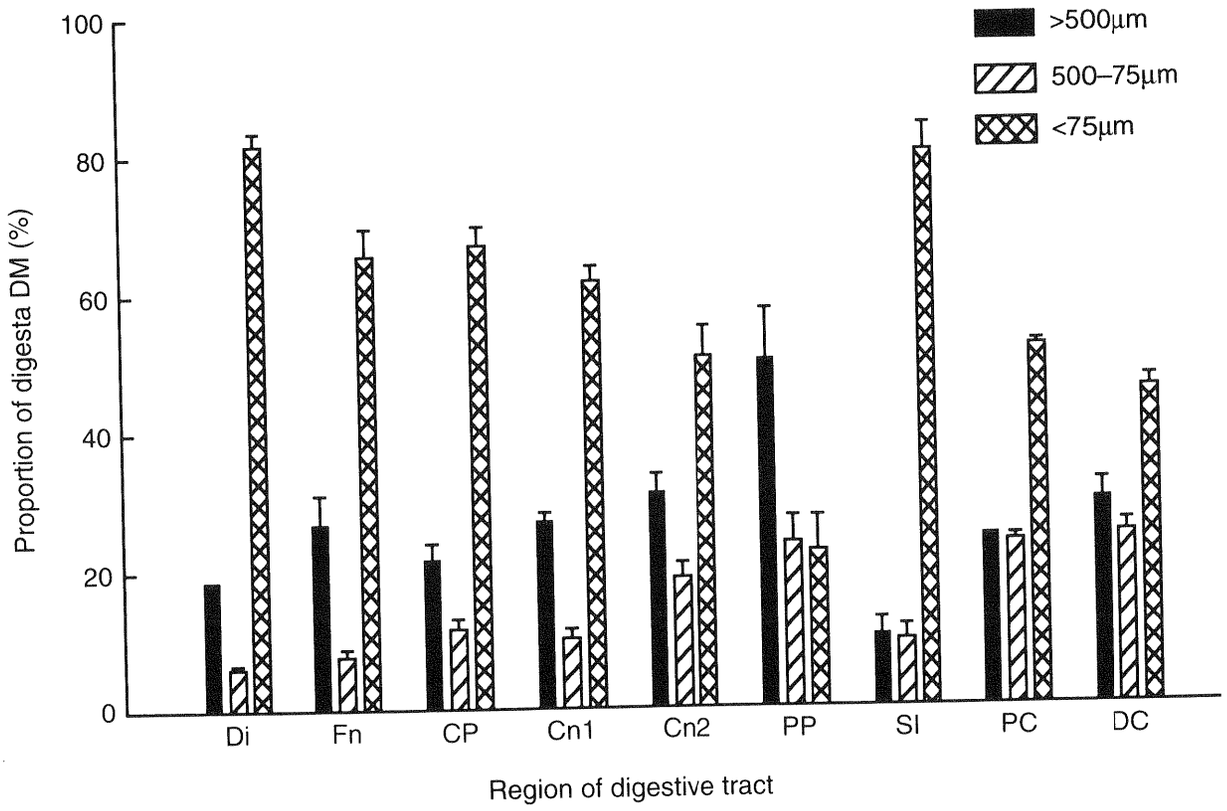

FIG. 6. The distribution of particles of three size classes in different regions of the digestive tract of *Bradypus tridactylus*. Abbreviations as per Figs 1 and 2. Mean ± S.E. ($n = 6$).

generally similar to those found in other foregut fermenters eating fibrous diets (Kuhn, 1964; Bauchop & Martucci, 1968; Ohwaki *et al.*, 1974; Dellow, Nolan & Hume, 1983; Hoppe, 1984) and in captive two-toed sloths eating fruit and vegetables. Denis *et al.* (1967) found total SCFA concentrations of 40–90 mM in the forestomach and 30–80 mM in the prepyloric stomach of *Choloepus hoffmanni*. However, in the present study, it was notable that there was a virtual absence of branched chain fatty acids (iso-butyric and iso-valeric) which, at least in ruminants, are derived from the fermentation of proteins. It is unlikely that fermentation in sloths is radically different and the absence of branched chain fatty acids may reflect a paucity of soluble protein, possibly through the actions of tannins. The accumulation of propionate and butyrate in samples collected from the rectum suggested that fermentation continued during the prolonged storage in this part of the gut. However, this digesta was poorly mixed and these SCFA are likely to be of little significance to the animal.

None the less, although total concentrations of SCFA were similar to those in other species, the rate of fermentation was particularly slow compared with other small foregut fermenters eating fibrous diets. For example, Hume (1977) measured SCFA production rates *in vitro* of between 40 and 52 $mmol.l^{-1}.h^{-1}$ in the forestomach of wallabies and 23 $mmol.l^{-1}.h^{-1}$ in the rumen contents of sheep. In colobid primates eating foliage and fruit, *in vitro* fermentation rates are typically between 20 and 30 $mmol.l^{-1}.h^{-1}$ (Kuhn, 1964; Bauchop & Martucci, 1968; Ohwaki *et al.*, 1974).

In vitro methods invariably underestimate *in vivo* fermentation rates, especially in foregut fermenters whose diets supply some easily fermentable substrates such as sugars and starches. Furthermore, the amount of time that elapsed between the death of the animal and the commencement of fermentation in the present study was longer than ideal. None the less, the methods used in this study closely followed those used in studies of macropodid marsupials and sheep (e.g. Hume, 1977) and so the data have comparative value.

The slow fermentation rates are most likely due to the lignified nature of *Cecropia palmata* foliage. Cork & Hume (1983) measured similar fermentation rates in the caecum of koalas fed highly lignified *Eucalyptus* leaf. Another factor contributing to the low fermentation rate may be the low and variable body temperature of sloths. Yahav & Buffenstein (1991) have shown that caecal fermentation in another heterothermic mammal, the naked mole rat *Heterocephalus glaber* is strongly influenced by temperature of the fermentation contents. None the less, it seems clear that fermentative digestion contributes little to the overall energy intake of *B. tridactylus*. This is consistent with low levels of glucose in the blood and intolerance to insulin exhibited by sloths (Bauchop, 1978).

Passage of digesta markers

The methods used to establish the kinetics of digesta in the gut were, necessarily, novel because sloths defecate so infrequently. Cumulative excretion curves were constructed from data from three different markers and from six animals. However, there is evidence that both the method of calculation and the pooling of results from different markers did not seriously compromise our conclusions.

Previous studies in domestic ruminants have shown that the three fluid phase markers and the three solid phase markers give similar estimates of passage rates and kinetics (Ellis *et al.*, 1982; Teeter & Owens, 1983). We found similar results for Co-EDTA and Cr-EDTA when dosed

together and for La and Sm but, through error, not all markers were given simultaneously. None the less, it is likely that the solid phase markers represent more the kinetics of fine particulate digesta than of coarse particles. Rare earth elements dosed in liquid form have a strong affinity for particulate digesta but may migrate between labelled and unlabelled particles (Hartnell & Satter, 1979; Ellis *et al.*, 1982). Given that fine particles probably move with solute digesta (Sakaguchi & Hume, 1991), it is perhaps not surprising that there was no difference between the retention time of solids and liquids in these experiments. Any future studies should try to bind particulate markers to pieces of food before dosing and examine the kinetics of particles of different sizes.

We are confident that our derivation of mean retention time (MRT) from composite excretion curves is valid because, in one animal, we were able to compare the results with calculations using traditional methods (Warner, 1981). This animal defecated seven times during the course of the experiment and we used the pattern of excretion of the first pair of markers dosed (Cr-EDTA and $YbCl_3$ at 216 hours before death) to calculate MRT. The MRT of these markers from ingestion to excretion was 157 h calculated by a total collection method (Warner, 1981) compared with the value of 147–152 h derived by measuring the area under the composite excretion curve. We concluded therefore that our calculation method was reasonable.

The passage of digesta through the gut of the three-toed sloth was slow compared to that observed in other small foregut-fermenters fed fibrous diets. For example, Dellow (1982) estimated MRTs of similar-sized macropodid marsupials of 15–25 h. In colobid primates (*Colobus guereza* and *Nasalis larvatus*) MRTs are between 25 and 30 h (Sakaguchi *et al.*, 1991; Dierenfeld, Koontz & Goldstein, 1992). However, an earlier study in sloths estimated that digesta were retained in the gut many times longer than we estimate. Montgomery & Sunquist (1978) fed free-living *Bradypus variegatus* glass beads, 3 mm in diameter and estimated 95% excretion times of about 50 days. Part of the difference may reflect the differences between free-living and captive animals and between the larger *B. variegatus* compared with *B. tridactylus* that we studied. However, we believe that the glass beads probably accumulated in the prepyloric stomachs, as did coarse digesta in this study. The 3 mm beads used by Montgomery & Sunquist (1978) were much larger than the digesta particles which were found in the prepyloric stomach and we suspect that this strongly influenced the ultimate retention time that they calculated.

None the less, given the irregular defecations of sloths, it is more important to identify the site of digesta retention rather than the overall retention time, since accumulation of digesta in the rectum is likely to be of less nutritional benefit to the animal even though it appeared that fermentation appears to continue during storage of faeces (Table I). Our data clearly show that the digesta were retained longest in the fermentative region of the stomach (that is cranial to the prepyloric stomach). It is here that fermentative digestion is most important. In contrast, retention of faeces in the rectum was only 17% of the overall mean retention time.

Marker appeared to disperse initially into the fundus and into the central and connecting pouch (Fig. 4). The consistency of marker and SCFA concentrations in the connecting and central pouches (Fig. 4 and Table I) suggests that this part of the gut forms a single, well-mixed pool of digesta. In contrast, marked digesta did not enter the diverticulum until about 24 h after dosing and had essentially left. Based on these observations, there appears to be little support for the view of Britton (1941) that this part of the gut serves to prolong retention of food. The single measurement of fermentation that we made in this part of the gut suggested similarities with the other parts of the stomach. At present, any special function of the diverticulum is not apparent.

Digesta particle size

The accumulation of coarse digesta particles in the prepyloric stomach was unexpected (Fig. 6). Langer (1988) observed a broadly similar pattern in one preserved museum specimen of *B. tridactylus* but also noted a large proportion of coarse particles in the connecting pouch. This was not corroborated in the present study. In ruminants, large particles are more resistant to flow from the reticulum via the reticulo-omasal orifice and, in camelids, large particles are selectively retained in the third forestomach compartment (Lechner-Doll, Kaske & Engelhardt, 1991).

In artiodactyls the large particles are retained until broken down either by rumination or by physico-chemical processes in the forestomach. It seems unlikely that this occurs in the sloth forestomach since the reflux of acidic digesta would not be favourable to further microbial activity. Alternatively, Britton (1941), upon noting the coarse and fibrous nature of the digesta in the prepyloric stomach, suggested that this part of the gut may function like a gizzard. While this part of the stomach does have very thick muscular walls, there are no structures similar to the grinding plates found in the gizzard of herbivorous birds.

In the two-toed sloth, *Choloepus hoffmanni*, the muscular part of the prepyloric stomach is lined with keratinized epithelium (Denis *et al.*, 1967). Presumably, this confers resistance to the strongly abrasive effect of the coarse digesta particles. Whether a similar pattern is found in *Bradypus tridactylus* is not yet known. We suggest that the accumulation of coarse digesta particles in the prepyloric stomach may simply represent the more rapid passage of fine particles from that part of the stomach rather than the selective retention of the coarse material.

Results from this study indicate that the digestive physiology of three-toed sloths differs in a number of aspects from that of true ruminants and that their description as 'ruminant-like' (e.g. Bauchop, 1978) should be discontinued. The areas of major difference identified in the present study is the lack of separation between the particulate and solute digesta phases and, in particular, the accumulation of coarse digesta particles in the prepyloric stomach.

Digestion in three-toed sloths is dominated by the slow passage and slow fermentation of a large volume of digesta in the forestomach. Although such a pattern could be expected to yield energy only very slowly, it is feasible because of the low-energy expenditures of free-living sloths (Nagy & Montgomery, 1980). None the less, we expect that such a strategy is restricted to sloths because, although the metabolic rates of other folivores are low, they do not approach the levels seen in *Bradypus* and *Choloepus*.

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